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Bioassimilable sulphur provides effective control of *Oidium neolycopersici* in tomato, enhancing the plant immune system †

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Abstract

BACKGROUND: Developments of alternatives to the use of chemical pesticides to control pests are focused on the induction of natural plant defences. The study of new compounds based on liquid bioassimilable sulphur and its effect as an inductor of the immune system of plants would provide an alternative option to farmers to enhance plant resistance against pathogen attacks such as powdery mildew. In order to elucidate the efficacy of this compound in tomato against powdery mildew, we tested several treatments: curative foliar, preventive foliar, preventive in soil drench and combining preventive in soil drench and curative foliar.

RESULTS: In all cases, treated plants showed lower infection development, better physiological parameters and a higher level of chlorophyll. We also observed better performance in parameters involved in plant resistance such as antioxidant response, callose deposition and hormonal levels.

CONCLUSION: The results indicate that preventive and curative treatments can be highly effective for the prevention and control of powdery mildew in tomato plants. Foliar treatments are able to stop the pathogen development when they are applied as curative. Soil drench treatments induce immune response mechanisms of plants, increasing significantly callose deposition and promoting plant development.

Keywords: bioassimilable sulphur; induced resistance; *Oidium neolycopersici*; *Solanum lycopersicum*

1 INTRODUCTION

Powdery mildews (Ascomycotina: Erysiphales) are among the most frequently encountered plant-pathogenic fungi worldwide, infecting leaves, stems, flowers and fruits of nearly 10 000 species of angiosperms. A broad range of economically important plants such as grapes, tree fruits, small grains, horticultural crops and many ornamentals are affected by them.[1] As they are obligate plant pathogens, powdery mildew species need a specific host to complete their life cycle. *Oidium neolycopersici* L. Kiss has been described worldwide as the causal agent of powdery mildew on tomato (*Solanum lycopersicum* L.), mainly in greenhouses, but it is also of increasing importance on field-grown tomato crops.[2–4] It causes typical powdery white lesions on the adaxial tomato leaf surface, also infecting abaxial surfaces, petioles and the calyx.[2–4] Severe infections lead to leaf chlorosis and premature senescence, resulting in considerable defoliation and a marked reduction in fruit size and quality.[2,5,6]

In general, research on disease control management has been especially focused on the development of procedures and chemical products to prevent or reduce the infection with preventive treatments. Traditionally, control of powdery mildews was only achieved by the use of active ingredients such as benomyl, bitertanol, bupirimate, carbendazim, fenarimol, pyrazophos, thiabendazol, triforine or various sulphur preparations.[3,7] Elemental sulphur (S^0) is probably the oldest of all pesticides and remains widely recognised as a valuable fungicide for the control of powdery mildews on fruit and vegetable crops. Sulphur has become attractive as a chemical control agent owing to its negligible toxicity to animals and beneficial insects and its low toxicity to plants, being also a common component of integrated pest management programmes. Elemental sulphur is non-systemic, and repeated applications are usually required to give an optimum protection.[8]

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Thus, a high input of S^0 is required for the control of powdery mildews, which may lead to environmental and economic losses for the growers.

Control of plant diseases still relies mainly on the use of synthetic fungicides, but growing concerns over the potential impact of them on human health and the environment have stimulated the search for alternative control strategies.[9–11] Therefore, for these reasons, as well as the danger of development of fungicide-resistant pests, research for compounds that could be a substitute for or reduce the input of chemicals in agriculture is required. Biological control by using antagonist microorganisms or biological-based products is one of the most important alternatives to chemicals in integrated and organic crop protection.[10] Currently, much attention is given worldwide to biological and integrated means of control of powdery mildews in greenhouse crops, yielding reports on many potential antagonists.[10,12] On the other hand, research focused on cultivar resistance to powdery mildews in important economically crops, such as tomato, has also been evaluated as a potential alternative tool.[3]

One of the most promising alternatives to the classical treatments is the induction of plant natural defences. It is well known that, under the proper stimuli, plants are able to activate a battery of responses that cope with the stress and usually are enough to overcome the threat and survive.[13] In the last years, several compounds have been studied for their ability to induce resistance in plants against different stresses either by direct activation of the defensive responses or by inducing the plants into a priming state.[14,15] Priming is a state of activation of plant defences that leads to a faster and stronger defensive response after pathogen attack. Some of these compounds, such as acibenzolar-*S*-methyl or hexanoic acid, have proved to be effective in herbaceous and woody crops against a wide range of pests.[16] Moreover, the low toxicity of these inducers and their persistent effect for several months makes them one of the most valuable alternatives to the classical pesticides. The resistance induced by priming agents is usually related to the activation of defensive responses, such as the upregulation of hormonal pathways related to defence, the accumulation of callose deposition or the activation of antioxidant machinery.

The study of new compounds based on liquid bioassimilable sulphur (LBS) (SO_3) and its effect as an inductor of the immune system of plants would provide an alternative option to farmers to enhance plant resistance against pathogen attack such as powdery mildew. Preliminary studies carried out in Valencia Province (eastern Spain) in both greenhouse and field conditions in different horticultural crops showed that the shoot and root development of plants treated with LSB was markedly higher than that observed in untreated plants. Moreover, less incidence of powdery mildews was also observed in treated plants (unpublished results). These preliminary results suggested that LBS may have systemic properties inside the plant, being well distributed through the vascular tissues. Moreover, we hypothesised that bioassimilable sulphur could be able to induce the activation of natural plant defences after treatments. However, no biochemical studies have been performed yet to demonstrate these hypotheses.

Therefore, the main goal of this study was to elucidate the efficacy of LSB in tomato against powdery mildew. To this end, *O. neolycopersici* has been used in combination with *S. lycopersium* as a model to study the biochemistry and physiology of plant–microbe–LSB interaction. Several treatments – (i) curative foliar, (ii) preventive foliar, (iii) preventive in soil drench, (iv) combining preventive in soil drench and curative foliar – were performed. Infection development, physiological parameters, the level of chlorophyll and the main parameters involved in plant resistance were monitored.

2 MATERIALS AND METHODS

2.1 Plant material and greenhouse conditions

Seeds of tomato cv. Montecarlo were sowed in plug trays with 96 cells filled with a mixture of 90% peat (Gramoflor GmbH & Co., Vechta, Germany) and 10% perlite (P.V.P Industries Inc., Bloomfield, OH). The plug trays were placed in a greenhouse with controlled temperature (18–24 °C). Three weeks after sowing, tomato seedlings were transplanted to plastic pots (1 L) filled with 500 g of peat–perlite mixture. Seedlings were grown under greenhouse conditions (18–24 °C) with a 16 h light photoperiod and watered every 3 days or as needed. The seedlings were maintained in the greenhouse of the Department of Agricultural Sciences of Universitat Jaume I of Castellon.

2.2 Fungal isolate and inoculum preparation

A representative isolate of *O. neolycopersici* (UJI1) collected from tomato cv. Ailsa grown in a commercial tomato orchard located in Castellon (eastern Spain) was used in this study. For inoculum conservation, a conidial suspension of *O. neolycopersici* isolate UJI 1 was prepared by washing the sporulating mycelium from freshly heavily infected leaves with tap water.[17] Subsequently, plants of tomato cv. Montecarlo were inoculated by spraying the conidial suspension. To induce sporulation on leaves, inoculated plants were incubated under plastic covers at 100% RH under greenhouse conditions (24 ± 3 °C) with a 16 h light photoperiod. For inoculum preparation, conidial suspensions were obtained from leaves of inoculated tomato cv. Montecarlo, which were 80–100% covered by fresh (8 days old) sporulating mycelium of *O. neolycopersici* isolate UJI 1, as described above. The resulting spore suspension was filtered to remove mycelia and plant fragments through two layers of cheesecloth into a 250 mL Erlenmeyer flask. The filtrate spore suspension was diluted with sterile distilled water (SDW) and adjusted with a haemocytometer to 10^5 conidia mL^{-1} and used immediately.

2.3 Effect of liquid bioassimilable sulphur (SO_3) on plant growth and the plant immune system

2.3.1 Liquid bioassimilable sulphur and treatments

Liquid bioassimilable sulphur (LBS) (bioabsorbable sulphur, SO_3 – 320 g L^{-1}, Naturdai S-System) was provided by the company Idai Nature SL (La Pobla de Vallbona, Valencia, Spain).

Treatments were performed when seedlings were at the 3–4-true-leaf stage by foliar applications and/or soil drench applications at 1.5 and 2.5 cm^3 L^{-1}, respectively, according to the manufacturer's instructions. The following treatments and combinations were tested: (i) foliar curative treatments applied when the first symptom of infection appeared after inoculation; (ii) preventive foliar treatments applied 2 days before inoculation and repeated once 15 days after the first treatment; (iii) preventive radicular treatments applied 2 days before inoculation and repeated once 15 days after the first treatment; (iv) combination of preventive radicular treatment applied 2 days before inoculation and curative foliar treatment applied when the first symptom of infection appeared after inoculation. Plants were inoculated with *O. neolycopersici* isolate UJI 1 as described above. Inoculation of all plants was made on the same day. Ten replicates (plants)

per treatment were used. In each experiment, ten non-treated and non-inoculated and ten non-treated and inoculated plants were included as negative and positive controls respectively. The experiment was repeated 3 times.

2.3.2 *Disease severity, plant growth and chlorophyll content evaluation*

To evaluate disease severity, individual mature leaves of each plant were evaluated 20 days after inoculation by scoring severity on a rating scale of 0–4, where 0 = no symptoms, 1 = ≤25% leaf surface covered with mycelium, 2 = 25–50% covered, 3 = 50–75% covered and 4 = ≥75% covered.

Total growth was measured on the last day of the experiment, measuring the plant length from soil surface to apical shoot. The chlorophyll level of the leaves of four-week-old tomato plants was measured using a chlorophyll meter (SPAD; Minolta, Tokyo, Japan). The three SPAD readings taken on one leaf for each of the ten plants per treatment were averaged to represent one observation. The results were obtained as SPAD values (arbitrary units).

2.3.3 *Determination and quantification of H_2O_2 and callose deposition evaluation*

Samples of ten leaves were collected for 3,3-diaminobenzidine (DAB) staining at the end of the experiment. Leaves were cut and put immediately in 1 mg mL^{-1} of DAB at pH < 3 for 24 h in the dark and were subsequently destained in 96% ethanol and rehydrated in distilled water. DAB staining intensities were quantified in micrographies by the number of dark-brown DAB pixels in relation to the total pixels corresponding to plant material using Adobe Photoshop CS4 software (Adobe Systems, Inc., San Jose, CA).

Callose deposition was determined, as described by Scalschi *et al.*,[18] in control and infected leaves at 20 days after inoculation. Leaves were collected and incubated in 95% ethanol at room temperature. Destained leaves were washed in 0.07 M phosphate buffer (pH 7), incubated for 15 min in 0.07 mM phosphate buffer containing 0.01% aniline blue at room temperature and then incubated in 0.1% aniline blue 1 week at room temperature. Observations were performed with an epifluorescence microscope. Callose deposition was quantified from digital photographs of aniline-blue-stained leaves. Fluorescence emitted by stained callose was observed under UV light as bright yellow spots and was analysed as the total number of pixels using Adobe Photoshop CS4 software. Callose intensity was expressed as the average of yellow pixels per million pixels in digital photography.

2.3.4 *Evaluation of hormones related to plant defence by chromatographic analysis*

For hormonal analysis, fresh material was frozen in liquid nitrogen (N, 0.5 g of the frozen tissue was homogenised in 2.5 mL of ultrapure water, and a mixture of internal standard {deuterated abscisic acid ([$^2H_6$] ABA), deuterated salicylic acid ([$^2H_4$] SA), dihydrojasmonic acid (dhJA) and propylparaben} was added at 100 ng mL^{-1} prior to extraction. After extraction, a 20 μL aliquot was injected directly into the high-performance liquid chromatography (HPLC) system. Analyses of hormone samples were carried out using a Waters Alliance 2690 HPLC system (Milford, MA) with a nucleosil ODS reversed-phase column (100 mm × 2 mm, i.d. 5 µm; Scharlab, Barcelona, Spain; http://www.scharlab.com). The chromatographic system was interfaced to a Quatro LC (quadrupole–hexapole–quadrupole) mass spectrometer (Micromass; http://www.micromass.co.uk). MASSLYNX NT software v.4.1 (Micromass) was used to process the quantitative data from calibration standards and plant samples.

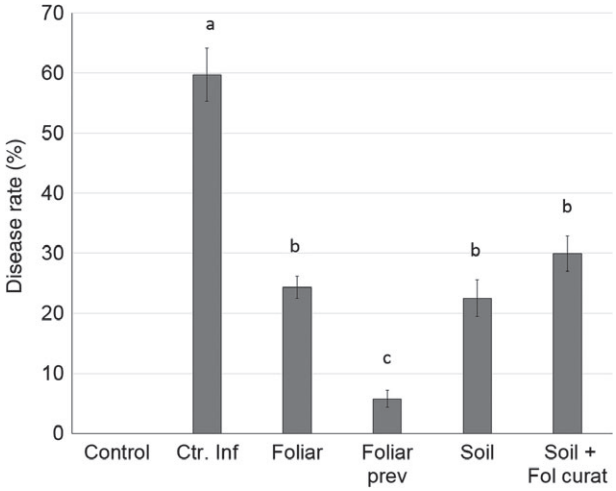

Figure 1. Effect of different treatments of liquid bioassimilable sulphur on tomato plants inoculated with *O. neolycopersici* isolate UJI1. The disease rate is expressed as percentage of leaves covered by the fungi for the treatments: (i) Control: uninfected and untreated plants; (ii) Ctr. Inf: infected and untreated plants; (iii) Foliar: foliar curative treatments; (iv) Foliar prev: foliar preventive and curative treatments; (v) Soil: soil drench preventive and curative treatments; (vi) Soil + Fol curat: soil preventive and foliar curative treatment. Vertical bars represent the average of ten plants ± standard error (SE), and different letters represent significant differences ($P \leq 0.05$, LSD test).

2.4 Statistical analysis

Statistical analysis was performed by using Statgraphics Centurion XVI software (Statpoint Technologies, Warrenton, VA). Data were submitted to ANOVA for population groups that followed a normal distribution, and the means were separated using Fisher's least significant difference (LSD) at 95%. It was considered that there were significant differences when $P < 0.05$.

3 RESULTS

3.1 Effect of LBS on disease severity, plant growth and chlorophyll content

All of the LBS treatments resulted in a significantly reduced disease ratio in comparison with untreated control plants 20 days after inoculation, showing a reduction in the symptoms in leaves of between 60 and 90%, depending on the treatments (Fig. 1). Foliar preventive treatment was the most effective, reducing 90.4% of the fungal infection in comparison with infected but not treated plants. Foliar curative and radicular preventive treatments showed similar effectiveness by reducing the disease symptoms by 59 and 62.2%. The treatment with lower effectiveness was the single radicular preventive treatment combined with a foliar curative application, reaching a reduction in the disease ratio of 49.8% compared with infected but not treated plants.

Treatments by soil drench showed an enhancement of plant growth (Fig. 2). Both treatments, preventive soil drench and preventive soil drench combined with curative foliar treatment, resulted in plants 13 cm higher than infected control plants. On the other hand, preventive and curative foliar applications resulted in plants 4 cm higher than infected controls, but without significant differences from uninfected control plants.

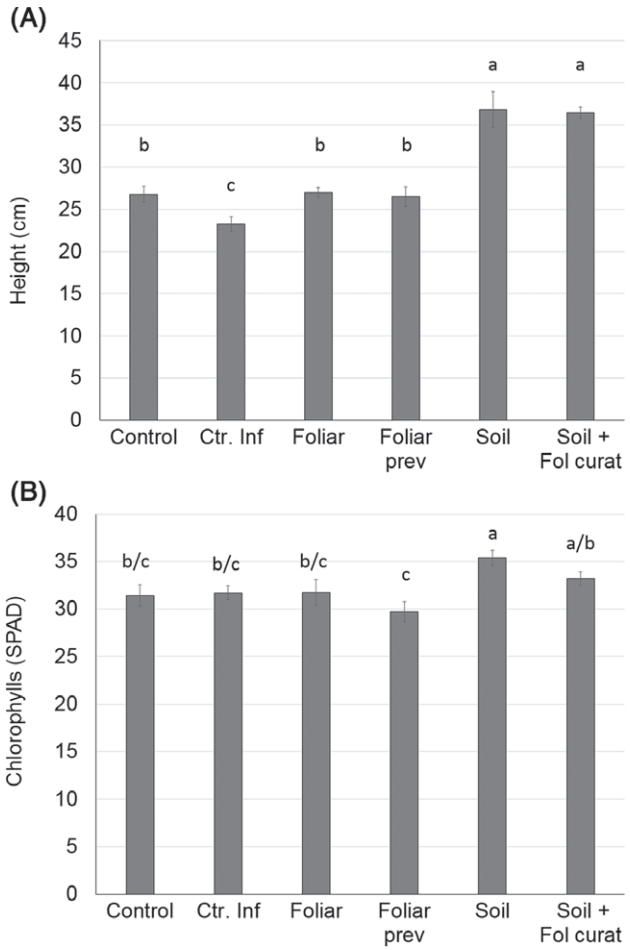

Figure 2. Effect of different LBS treatments on physiological parameters measured 30 days after treatment: (A) plant height measured from soil to apical shoot; (B) chlorophyll content expressed in SPAD units (dimensionless). Treatments are the same as those in Fig. 1. Vertical bars represent the average of ten plants ± SE, and different letters represent significant differences ($P \leq 0.05$, LSD test).

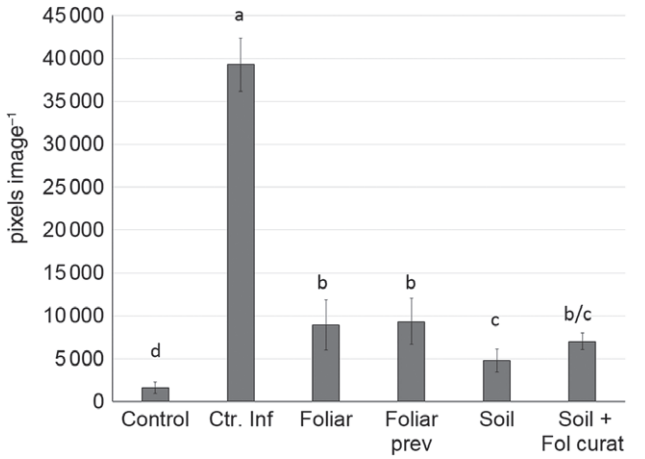

Figure 3. H_2O_2 staining, estimated by using DAB staining in the leaves of infected tomato plants. Treatments are the same as those in Fig. 1. Vertical bars represent the average of ten plants ± SE, and different letters represent significant differences ($P \leq 0.05$, LSD test).

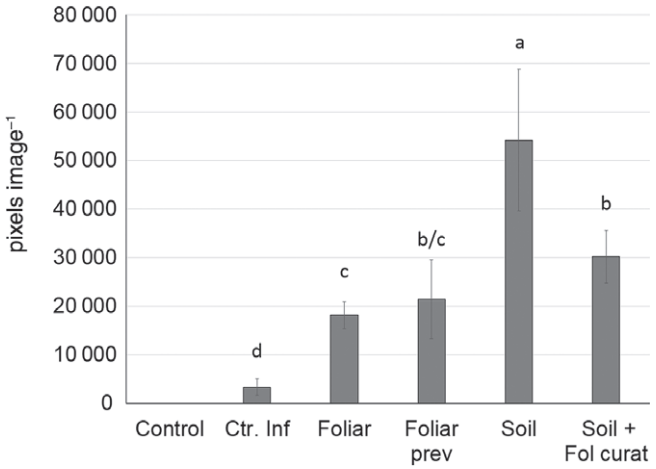

Figure 4. Quantification of callose deposition after different treatments of LBS. Treatments are the same as those in Fig. 1. Vertical bars represent the average of ten plants ± SE, and different letters represent significant differences ($P \leq 0.05$, LSD test).

Chlorophyll levels showed significantly higher values when soil treatments were used. Only preventive soil drench showed significantly higher values for this parameter, resulting in an increment of 20% compared with the rest of the treatments. However, foliar treatments or punctual soil treatments did not show significant differences with infected or uninfected controls.

3.2 Effect of LSB on the levels of H_2O_2 and on callose deposition

The accumulation of H_2O_2 in response to *O. neolycopersici* was determined by using DAB staining. The H_2O_2 that accumulates at the infection sites reacts with the DAB to produce a dark brown insoluble precipitate. Infected plants treated with LSB exhibited fewer dark brown pigments, indicating a reduction of H_2O_2. As shown in Fig. 3, the reduction achieved was nearly 95% when the compound was applied by soil drench treatments, and 75% when the compound was applied by foliar treatments.

To assess the ability of the treatment to induce defensive responses, we studied the effect of the treatment on callose deposition. Results obtained showed that all the treatments were able to enhance callose accumulation upon infection (Fig. 4). Soil preventive treatment was the most effective, showing an accumulation 16-fold higher than infected control plants, whereas the soil preventive and foliar curative reached an accumulation of callose ninefold higher than infected controls. On the other hand, foliar treatments showed an accumulation only five- and sixfold higher for curative and preventive treatments respectively.

3.3 Effect of LBS on the enhancement of hormones related to plant defence

The hormonal changes in treated and infected plants were analysed in order to ascertain the mechanisms induced by the treatment (Fig. 5). Foliar treatment showed an induction of salicylic acid (SA), whereas in both soil drench treatments the levels of this hormone were even lower than in untreated and infected controls. On the other hand, drench soil preventive treatment resulted in a significant enhancement of jasmonic acid (JA). Moreover, the response of abscisic acid (ABA) to the treatment was similar to that observed in the JA, showing significantly higher levels in soil-drench-treated plants.

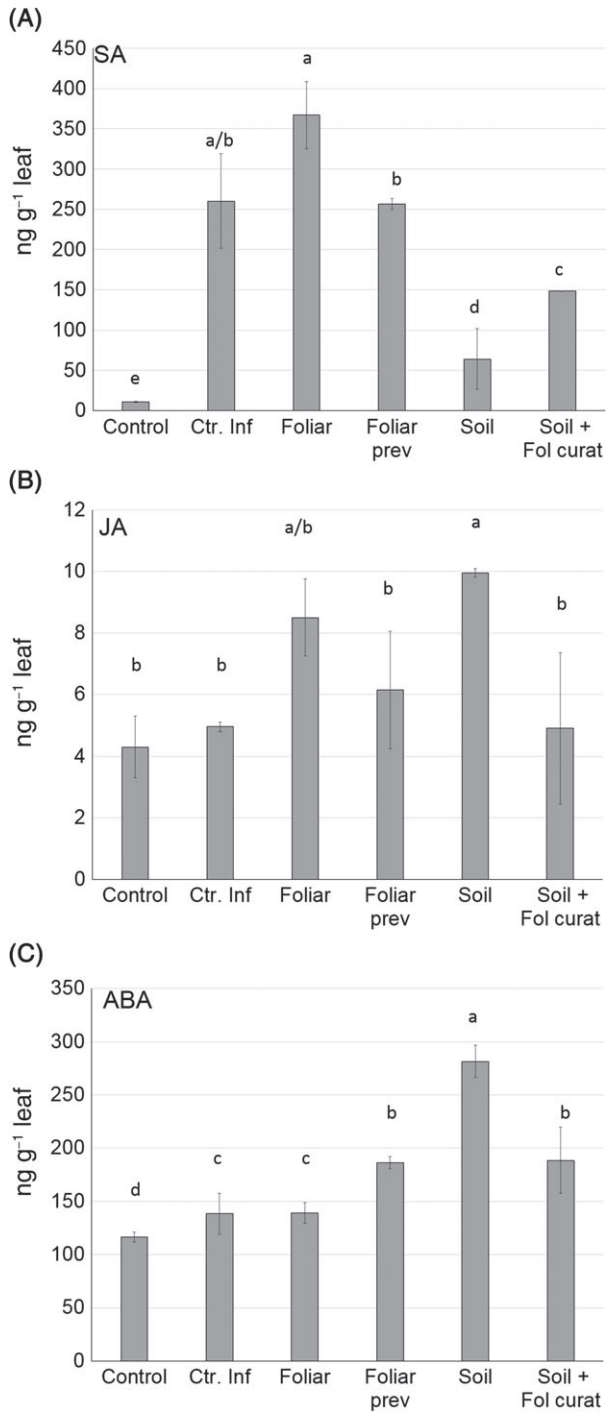

Figure 5. Hormone levels in infected and in treated and infected tomato plants after different treatments with LBS. The SA (A), JA (B) and ABA (C) levels were determined in freeze-dried material by HPLC. Treatments are the same as those in Fig. 1. Vertical bars represent the average of ten plants ± SE, and different letters represent significant differences ($P \leq 0.05$, LSD test).

4 DISCUSSION AND CONCLUSIONS

The efficacy of sulphur as a fungicide in common agriculture was discovered in the early nineteenth century. Since then, treatment with sulphur dust, sulphates or sulphur derivatives has become usual practice.[19] Nowadays, sulphur and its derivatives are still listed among the recommended products to protect tomato crop against different fungal pathogens, such as powdery mildews. In the present work, we have analysed the effectiveness of LBS against *O. neolycopersici* in tomato plants. We have demonstrated that, besides the well-known antifungal effect of the sulphur compounds, the bioassimilable formulation is able to induce the natural defensive responses of the plants.

The suggested mode of action of S^0 as an antifungal compound relies on the permeability of fungal cells. This element is taken up into the cytoplasm, where it affects the oxidation state of the respiratory complexes, disturbing the electron flux in the mitochondrial respiratory chain, which results in the well-known fungitoxicity.[8] However, our results showed better protection against *O. neolycopersici* when the compound was applied as a preventive soil drench, suggesting that the protective effect of the bioassimilable sulphur may be related to the induction of plant defences. Previous studies have suggested that the application of sulphur can improve the natural resistance of plants against fungal pathogens through the stimulation of metabolic processes that involve S^0, resulting in so-called sulphur-induced resistance (SIR).[20]

Callose accumulation is a characteristic cellular response of early post-invasive defences that prevents the colonisation of the pathogen by creating a physical barrier at the site of the infection. This barrier is able to slow pathogen invasion in the attacked tissue, giving more time to activate additional defence responses that may require gene activation and expression.[21] In this way, Ellinger *et al.*[22] showed that elevated early callose deposition leads to complete penetration resistance to several powdery mildews. It has also been demonstrated that some inducers of resistance in plants are able to enhance callose deposition.[23] Moreover, this induced response is directly related to the level of resistance achieved by the plant after priming treatments.[24–27] Our results showed an enhancement of callose deposition in treated plants, especially in those treated by soil drenches, which could indicate that the protective effect is due to an induction of plant defences.

In order to ascertain the implication of the defensive pathways in the enhancement of resistance induced by the compound, the hormonal pathways were analysed. Interestingly, different results were obtained, depending on the mode of application of the product or treatment. Whereas foliar spray resulted in slightly higher levels of SA, soil drench applications resulted in an enhancement of JA, and the accumulation of SA was repressed. SA levels usually increase in response to pathogen infection, inducing the expression of pathogenesis-related (PR) genes and resulting in an enhancement of resistance that usually is effective against biotrophic fungi.[28] However, Achuo *et al.*[29] demonstrated that the SA defence pathway is able to induce resistance in tomato against *Botrytis cinerea* Pers. but not against *O. neolycopersici*. JA is a hormone able to induce several defence responses. It is generally accepted that JA-dependent defences are activated by necrotrophic fungi and wounding insects. However, some studies have demonstrated that certain biotrophic fungi can trigger the activation of JA-dependent responses.[30] It has also been demonstrated that the application of JA increases resistance to *O. neolycopersici* in tomato.[31] Our results showed that the levels of ABA are strongly enhanced in soil-drench-treated plants. This result agrees with previous literature, which reported a synergistic effect between ABA and JA and the upregulation of callose deposition, suggesting a general role of ABA in modulating biotic stress-induced JA responses.[26–28,32] According to our results, ABA may be acting as a signal to enhance callose deposition in SIR, as

higher levels of callose deposition observed in soil-drench-treated plants correlate with higher levels of ABA in the same plants.

On the other hand, plant defence usually requires compounds that contain sulphur, such as antimicrobial peptides phytoalexins, thionins or defensins, coenzyme A (as a precursor of SA) or glutathione.[20] Glutathione plays different roles in plant defence, such as detoxification of herbicides or a precursor of phytochelatines, which are involved in heavy metal detoxification.[33] However, the most important role of this molecule is its ability as a redox buffer. During pathogen attack, reactive oxygen species (ROS) can be produced by plant cells via the enhanced enzymatic activity of plasma-membrane-bound NADPH-oxidases as well via disruption of cellular homeostasis, producing oxidative damage in membrane lipids, nucleic acids and proteins.[34] At the same time, the activity and levels of the ROS-detoxifying enzymes APX and CAT can be suppressed by SA, producing more ROS while lowering its ROS-scavenging capacities.[35] It has been demonstrated that high concentrations of glutathione would confer better antioxidant responses, which in certain tomato species correlates with salt tolerance.[34] Our results showed that in all treated plants peroxide levels in leaves were 4 times lower than in infected control plants, suggesting that the antioxidant machinery could be enhanced by the treatment.

Besides all the above, sulphur is a major nutrient essential for plant growth that is present in the amino acids and regulates photosynthesis by affecting the electron transport system. Its deficiency can reduce the chlorophyll and Rubisco content and the photosystem II efficiency.[36] Treated plants showed an enhancement of plant growth as well as chlorophyll content when the compound was applied by soil drench, resulting in plants 10 cm higher than uninfected healthy plants. This result suggests that the application of LBS not only is able to protect the plants through induction of natural defences but also improves the physiological performance of the plants.

In conclusion, our results reinforce the hypothesis that treatment with LBS either by foliar spray or by soil drench applications can be effective in controlling the pathogenic fungus *O. neolycopersici* in tomato plants. Data suggest that this compound is able to induce resistance in tomato plants by callose deposition, modification of hormonal pathways and ROS scavenging. Moreover, treated plants exhibit higher size and chlorophyll content, suggesting that the defensive responses induced by the bioassimilable sulphur do not have excessive energetic costs. Thus, these results are interesting from the viewpoint of obtaining alternatives to chemicals in crop protection in an integrated disease management strategy.

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REFERENCES

1 Glawe DA, The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annu Rev Phytopathol* **46**:27–51 (2008).
2 Chelal J and Hau B, Temporal Dynamics of Powdery Mildew (*Oidium neolycopersici*) and its effects on the host growth dynamics of tomato. *J Phytopathol* **163**:711–722 (2015).
3 Jones H, Whipps JM and Gurr SJ, The tomato powdery mildew fungus *Oidium neolycopersici*. *Mol Plant Pathol* **2**:303–309 (2001).
4 Whipps JM, Budge SP and Fenlon JS, Characteristics and host range of tomato powdery mildew. *Plant Pathol* **47**:36–48 (1998).
5 Lebeda A, Mieslerová B, Petřivalský M, Luhová L, Špundová M, Sedlářová M *et al.*, Resistance mechanisms of wild tomato germplasm to infection of *Oidium neolycopersici*. *Eur J Plant Pathol* **138**:569–596 (2014).
6 MieslerovÁ B, Lebeda A and Kennedy R, Variation in *Oidium neolycopersici* development on host and non-host plant species and their tissue defence responses. *Ann Appl Biol* **144**:237–248 (2004).
7 Mieslerová B and Lebeda A, Taxonomy, distribution and biology of the tomato powdery mildew (*Oidium lycopersici*). *J Plant Dis Prot* **106**:140–157 (1999).
8 Williams JS and Cooper RM, The oldest fungicide and newest phytoalexin – a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol* **53**:263–279 (2004).
9 Dayan FE, Cantrell CL and Duke SO, Natural products in crop protection. *Bioorg Med Chem* **17**:4022–4034 (2009).
10 Elad Y, Malathrakis NE and Dik AJ, Biological control of *Botrytis*-incited diseases and powdery mildews in greenhouse crops. *Crop Prot* **15**:229–240 (1996).
11 Flors V, Miralles MC, Varas E, Company P, Gonzalez-Bosch C and Garcia-Agustin P, Effect of analogues of plant growth regulators on *in vitro* growth of eukaryotic plant pathogens. *Plant Pathol* **53**:58–64 (2004).
12 Pertot I, Zasso R, Amsalem L, Baldessari M, Angeli G and Elad Y, Integrating biocontrol agents in strawberry powdery mildew control strategies in high tunnel growing systems. *Crop Prot* **27**:622–631 (2008).
13 Conrath U, Priming of induced plant defense responses. *Adv Bot Res* **51**:361–395 (2009).
14 Bektas Y and Eulgem T, Synthetic plant defense elicitors. *Front Plant Sci* **5**:804 (2015).
15 Aranega Bou P, Leyva MdlO, Finiti I, García Agustín P and González Bosch C, Priming of plant resistance by natural compounds. Hexanoic acid as a model. *Front Plant Sci* **5**:488 (2014).
16 Gozzo F and Faoro F, Systemic acquired resistance (50 years after discovery): moving from the lab to the field. *J Agric Food Chem* **61**:12 473–12 491 (2013).
17 Huang CC, Meijer-Dekens F and Lindhout P, Characterization and mapping of resistance to *Oidium lycopersicum* in two *Lycopersicon hirsutum* accessions: evidence for close linkage of two Ol-genes on chromosome 6 of tomato. *Heredity* **85**:511–520 (2000).
18 Scalschi L, Llorens E, Camañes G, Pastor V, Fernández-Crespo E, Flors V *et al.*, Quantification of callose deposition in plant leaves. *Bio-protocol* **5**(19) (2015).
19 Rusell PE, A century of fungicide evolution. *J Agric Sci* **143**:11–25 (2005).
20 Bloem E, Haneklaus S and Schnug E, Milestones in plant sulfur research on sulfur-induced-resistance (SIR) in Europe. *Front Plant Sci* **5**:779 (2014).
21 Voigt CA, Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Front Plant Sci* **5**:168 (2014).
22 Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C *et al.*, Elevated early callose deposition results in complete penetration resistance to powdery mildew in *Arabidopsis*. *Plant Physiol* **161**:1433–1444 (2013).
23 Franco F and Iriti M, Callose synthesis as a tool to screen chitosan efficacy in inducing plant resistance to pathogens. *Caryologia* **60**:121–124 (2007).
24 Abouraïcha E, El Alaoui-Talibi Z, El Boutachfaiti R, Petit E, Courtois B, Courtois J *et al.*, Induction of natural defense and protection against *Penicillium expansum* and *Botrytis cinerea* in apple fruit in response to bioelicitors isolated from green algae. *Sci Hort* **181**:121–128 (2015).
25 Llorens E, Fernandez-Crespo E, Vicedo B, Lapena L and Garcia-Agustin P, Enhancement of the citrus immune system provides effective resistance against *Alternaria* brown spot disease. *J Plant Physiol* **170**:146–154 (2013).
26 Ton J and Mauch-Mani B, Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J* **38**:119–130 (2004).

27 Vicedo B, Flors V, Leyva MD, Finiti I, Kravchuk Z, Real MD *et al.*, Hexanoic acid-induced resistance against *Botrytis cinerea* in tomato plants. *Mol Plant–Microbe Interact* **22**:1455–1465 (2009).
28 Pieterse CMJ, Van Der Does D, Zamioudis C, Leon-Reyes A and Van Wees SCM, Hormonal modulation of plant immunity. *Annu Rev Cell Dev Biol* **28**:489–521 (2012).
29 Achuo EA, Audenaert K, Meziane H and Hofte M, The salicylic acid-dependent defence pathway is effective against different pathogens in tomato and tobacco. *Plant Pathol* **53**:65–72 (2004).
30 Antico CJ, Colon C, Banks T and Ramonell KM, Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. *Front Biol* **7**:48–56 (2012).
31 Thaler JS, Owen B and Higgins VJ, The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiol* **135**:530–538 (2004).
32 Fan J, Hill L, Crooks C, Doerner P and Lamb C, Abscisic acid has a key role in modulating diverse plant–pathogen interactions. *Plant Physiol* **150**:1750–1761 (2009).
33 Leustek T, Martin MN, Bick J-A and Davies JP, Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annu Rev Plant Physiol Plant Mol Biol* **51**:141–165 (2000).
34 Mittler R, Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* **7**:405–410 (2002).
35 Apel K and Hirt H, Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* **55**:373–399 (2004).
36 Lunde C, Zygadlo A, Simonsen HT, Nielsen PL, Blennow A and Haldrup A, Sulfur starvation in rice: the effect on photosynthesis, carbohydrate metabolism, and oxidative stress protective pathways. *Physiol Plant* **134**:508–521 (2008).

